Revised 3/4/03

Transplantation Immunology

Developed by

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Note to Instructors

This workbook is divided into five sections:

1. Introduction to the POPS System, introduction to and objectives of the clinical simulation, and a pretest
2. Color-coded booklets with pretest answers and the clinical problem
3. Group question and answer sheets
4. Posttest
5. Posttest answers

Each student should receive a copy of the first section to study and answer questions before the group problem-solving session. If you wish, the second section also may be distributed for the students to review prior to the group session.

Transplantation Immunology

Pretest

1. Bone marrow failure is unlikely to be associated with:
   A. anemia
   B. increased susceptibility to infection
   C. leukopenia
   D. reticulocytosis
   E. thrombocytopenia

2. Which one of the following has **not** been used as a source of hematopoietic stem cells?
   A. Blood
   B. Bone marrow
   C. Cord blood
   D. Liver

3. The ideal donor of a hematopoietic stem cell transplant is:
   A. a close friend
   B. a first cousin
   C. a parent
   D. a sibling
   E. an identical twin

4. Hematopoietic stem cells are given to the recipient by:
   A. infusing the cells into the patient's bone marrow cavities
   B. infusing the cells into the patient's heart
   C. infusing the cells into the patient's blood
   D. infusing the cells into the patient's peritoneal cavity
   E. All of the above

5 Patients who have myelogenous leukemia are prepared for a hematopoietic stem cell transplant by:
   A. chemotherapy and radiotherapy
   B. laparoscopic surgery
   C. splenectomy
   D. thymectomy
   E. none of the above

6. Which of the following is an unlikely complication of hematopoietic stem cell transplantation?
   A. Failure of the graft to survive
   B. Graft-versus-Host Disease (GVHD)
   C. Infection
   D. Malignant transformation of the transplanted stem cells
   E. Treatment-associated malignancies

Pretest (ctd.)

7. The cells infiltrating target tissues in chronic graft-versus-host disease are:
   A. mixtures of mononuclear cells and granulocytes of donor origin
   B. mixtures of mononuclear cells of donor and recipient origin
   C. mixtures of mononuclear cells of donor origin and granulocytes of host origin
   D. mononuclear cells of donor origin
   E. mononuclear cells of host origin

8. Survival of a hematopoietic stem cell transplant and its establishment in the bone marrow may be determined by:
   A. complete blood cell counts
   B. evidence of chimerism
   C. liver biopsy
   D. lymph node biopsy
   E. measuring creatinine clearance

9. In the weeks following hematopoietic cell transplantation most patients require:
   A. folic acid megadoses
   B. interleukin-2 therapy
   C. psychotherapy
   D. red blood cell transfusions
   E. tetanus immunization

10. A risk associated with being a hematopoietic stem cell transplant **donor** is:
   A. anemia
   B. bone marrow failure
   C. infection
   D. leukemia
   E. lymphadenopathy

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BOOK A

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Transplantation Immunology

Learning Objectives

1. List some of the diseases for which allogeneic hematopoietic stem cell transplantation (HSCT) or allogeneic bone marrow transplantation (ABMT) is used as a therapy.
   A. Describe the sources of hematopoietic stem cells.
   B. Describe how a stem cell donor is chosen.

2. Describe how the outcome (success, failure) of an HSCT is determined.
   A. Define the goals of HSCT transplantation.
   B. Describe how the corrective effect of an HSCT is determined.

3. Describe the immunological barriers that must be considered in doing an HSCT.
   A. Describe host-versus-graft disease (HVGD) and graft-versus-host disease (GVHD).
   B. Describe how an immune response can have a detrimental effect on the success of an HSCT.
   C. Explain how the immune system of the graft recipient can be manipulated to prevent GVHD.
   D. Explain how a graft-versus-leukemia reaction can be of benefit to the patient.

4. Describe the risks associated with HSCT.
   A. Know the success rate of HSCT in curing a disease such as chronic myelogenous leukemia.
   B. Describe the possibility of recurrent disease, infection, cancer, and GVHD and how these conditions may affect the success of the HSCT.

5. Describe the pathogenesis and clinical manifestations of GVHD.

Transplantation Immunology

Booklet A
Pretest Correct Answers

1. (D) is correct. Anemia or a deficiency in red blood cells, leukocytopenia or a deficiency of white blood cells, and thrombocytopenia or a deficiency in platelets all may result from the failure of the bone marrow to produce these blood elements. Clearly, as a result of failure to produce the white blood cells, patients have increased susceptibility to infection. Thus, A, B, C, and E are all correct. Reticulocytosis, i.e. the entry into the peripheral circulation of immature red cells, is not seen when bone marrow failure is the cause of anemia, because the generation of red cells and their precursors is impaired.

5. (A) is correct. An important part of the preparation for a hematopoietic stem cell transplant is to kill off many, if not all, of the abnormal cancerous or bone marrow cells in the patient and to suppress the immune system of the host to avoid rejection of the transplant. This is accomplished by a series of treatments with chemotherapeutic drugs and total body irradiation, which eliminate most of the malignant cells (their rapid rate of proliferation makes them more susceptible to cytotoxic agents and irradiation). The same interventions ablate the patient's immune system. Surgical procedures such as removal of the spleen or thymus or other invasive procedures are not part of the preparation for bone marrow transplantation.

Transplantation Immunology

Introduction

Thousands of transplants of organs, tissues, and cells are performed throughout the world annually. Transplantation of bone marrow cells or hematopoietic stem cell transplantation (HSCT) is performed to correct failures in the production of red or white cells, to repair immune deficiencies, and as a means of replacing malignant cells which have taken over the bone marrow. The major barrier to organ, tissue, and cell transplantation in humans is the immune response to the graft or, in the case of HSCT, the immune response of the graft against the recipient.

This POPS leads to a discussion on the indications and problems associated with HSCT or allogeneic bone marrow transplantation (ABMT) in a patient with acute leukemia. Approximately 70% of the patients undergoing ABMT for the treatment of certain types of leukemia live at least five years. A major barrier to the success of HSCT is the fact that the patient's immune system can reject the donor stem cells or that lymphocytes in the HSCT will attack (reject) the recipient. Donor selection is very important in order to prevent or minimize immune reactions of host against graft and graft against host. It is important to find the best possible histocompatibility match between the donor and the patient.

There are at least seven genes in the major histocompatibility complex (MHC), located on chromosome 6, which code for HLA antigens. Each of these seven genes is polymorphic meaning that there are many different versions of each gene which differ from the others by a different sequence of nucleotides. Each of these gene variants, called **alleles**, code for an antigenically different HLA antigen. In the human population there are many, many thousands of different MHC gene combinations or **genotypes**, as they are called. Faced with this huge variability, it is very difficult, but not impossible, to find two human beings who have identical HLA antigens by searching in the population at random. Since HLA antigens are genetically determined, it is often possible to find individuals who are HLA identical, or matched, within a family.

The HLA antigens that receive the most attention are HLA-A, HLA-B and HLA-DR, however, HLA-C and HLA-DQ may also play an important role. Since each person carries a chromosome six of maternal and paternal origin there are two copies of each HLA gene all of which are expressed (co-dominant expression). Therefore, a “perfect” match is called a six-antigen match (2 HLA-A, 2 HLA-B, and 2 HLA-DR), although as previously stated there are other antigens that may be important. The HLA genes on a particular chromosome 6 constitute a “haplotype” (derived from haploid genotype). Each person has a maternal and a paternal haplotype based on which of the parent’s chromosome 6s were inherited.

The most likely place to find an HLA match between two people is among full-blooded siblings. According to Mendelian inheritance principles, one has a 25% chance of inheriting the same HLA molecules as a sibling, a 25% chance of inheriting none of the same HLA molecules as a sibling and a 50% chance of inheriting half of the same HLA molecules as a sibling. When HSCT is considered, the first thing is to do a “family study”. The patient and all available siblings and parents are HLA typed. This allows for identification of potential “six-antigen” matches among the siblings and analysis of the segregation patterns of the HLA molecules. The segregation patterns are obtained from determining which of the parental haplotypes are inherited. Each sibling from a given couple should have one haplotype from each parent.

Transplantation Immunology

Introduction (ctd.)

In the example shown below, five of the seven MHC genes of the two families were determined. Each HLA antigen coded for by an MHC gene is identified by a number. Since we inherit one set of genes from each of our parents, we all have two versions of each MHC gene and thus there are two numbers given for each gene. Because data from both parents and two siblings was available, it was possible to establish the genotypes for the different family members. Discuss the interpretation of the HLA typing data below within the group before turning this page.

HLA Genotypes

		Family 1					Family 2				
Family Member	HLA Gene	A	B	C	DR	DQ	A	B	C	DR	DQ
Mother		1	5	45	10	21	15	25	40	51	55
		2	6	46	11	22	16	26	41	52	56
Father		3	7	47	12	23	17	27	42	53	57
		4	8	48	13	24	18	28	43	54	58
Child 1		1	5	45	10	21	15	26	40	52	55
		4	8	48	13	24	17	27	43	53	57
Child 2		2	6	46	11	22	15	26	40	52	55
		3	7	47	12	23	17	27	43	53	57

Introduction (ctd.)

The family members in Family 1 are different from each other and there is no HLA match in the family. However, in Family 2 it is apparent that Child 1 and Child 2 are HLA identical. These examples are shown to make a point about the inheritance of MHC genes and the possibility of finding HLA identical individuals within families. If a sibling or other relative is thought to be a potential match, further testing is done to ensure that the person is the best match possible. This is called "higher-resolution" testing, and is performed by DNA testing utilizing a polymerase-chain reaction (PCR) based assay system.

If a match cannot be found within a patient's family, a search for a matched, unrelated donor can be performed through the National Marrow Donor Program (NMDP). The NMDP has a computerized list of people who have agreed to be stem cell donors and their HLA types. Currently, the NMDP has over 4 million people in its registry. Although this sounds like a lot, it must be realized that there are hundreds of thousands to hundreds of millions of different combinations of HLA genes, depending on the number of HLA loci considered. To complicate matters even further, certain HLA antigens are found in greater proportions among members of different racial groups. To put it another way, an African-American patient has a greater chance of matching certain HLA antigens with another African-American. Unfortunately, African-Americans are greatly underrepresented in the NMDP registry thereby decreasing the likelihood of a match for that ethnic group. The same could be said for most minority groups in this country.

Transplantation Immunology

Data Sheet A

Case History

A 43-year-old man is referred to the local hematologic/oncologic group by a general practitioner who, upon conducting a routine examination including a complete blood count (CBC), discovered that the white blood cell (WBC) number was elevated to 36,000 cells/µL (normal range 4,000-10,000 cells µl). The patient was in apparent good health with no specific complaints and the results of all other tests fell within normal limits.

The general practitioner had requested further analysis of the blood cells. It was found that the WBC number was reproducibly elevated and that differential cell analysis revealed that 85% of the white cells were neutrophils and 3% were basophils. Examination of a blood smear by a hematopathologist revealed that the majority of WBCs were immature neutrophils.

After consulting with the members of your group practice you perform a bone marrow biopsy and submit it to a pathologist for analysis. The marrow biopsy was reported to be hypercellular and an analysis of the chromosomes of dividing cells revealed that the Philadelphia chromosome was present.

Please discuss the following points:

1. What is the significance of detecting the Philadelphia chromosome?

2. What is the differential diagnosis in this patient?

3. What are the management options for this patient?

Data Sheet A

Comments

The Philadelphia chromosome is a small chromosome that is the result of a translocation or movement of part of the long arm of chromosome 22 to another chromosome, often chromosome number 9. Its presence is confirmatory of a diagnosis of chronic myelogenous leukemia (less than 10% of CML patients lack the Philadelphia chromosome). The identification of the Philadelphia chromosome in CML patients has prognostic value because patients who have this chromosome respond to chemotherapy and survive beyond 40 months, whereas Philadelphia chromosome negative patients respond poorly to chemotherapy and usually succumb to the disease within 15 months.

Leukemia can be thought of as a malignant neoplasia of white blood cell precursors (hematopoietic) or stem cells. The normal bone marrow of these patients is diffusely replaced with proliferating leukemic cells and abnormal, immature cells appear in the blood and infiltrate the liver, spleen, lymph nodes, and other sites in the body. The elevated white blood cell count in these patients is not fatal. Rather CML patients suffer from anemia, thrombocytopenia, and loss of normally functioning leukocytes because their bone marrow is heavily infiltrated by proliferating leukemic cells and the normal elements are few in number, unable to maintain a normal supply of normal cells. The infiltration by leukemic cells of many tissues and organs leads to widespread organ dysfunction and ultimately death if the leukemia is not aggressively treated.

Therefore, treatment options the age range of the patient presented here are represented by two quite different approaches. One approach is the treatment of the patient with imatinib mesylate (Gleevec), a selective inhibitor of the Bcr-Abl tyrosine kinase that is overactive in the malignant cells in CML. Gleevec can induce hematologic remissions in up to 95% of the patients and cytogenetic remissions in about 60% of the patients. The alternative treatment is hematopoietic stem cell transplantation (HSCT). These two treatment options are presented to the patient and the patient opted for treatment with Gleevec.

The next step is to hear about the follow-up of this patient.

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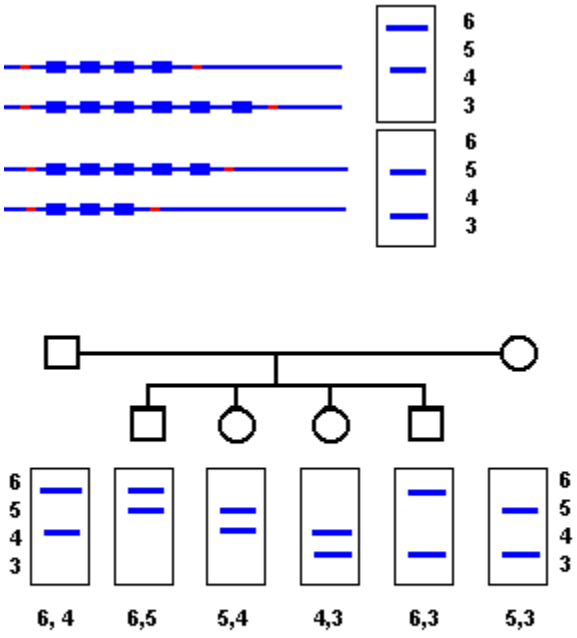
Booklet B
Pretest Correct Answers

2. (D) is correct. The blood, bone marrow, and cord blood connecting a fetus to the maternal body are all sources of hematopoietic stem cells. The liver is only involved in hematopoiesis very early during embryonic life (between weeks 6 and 20 of gestation) and has not been used as a source of stem cells.

8. (B) is correct. There are two possible favorable outcomes of a bone marrow or hematopoietic stem cell transplant: replacement of the recipient's bone marrow by the donor's bone marrow, or full recovery of autologous normal bone marrow function. Either outcome is commonly associated with chimerism, i.e., co-existence of cells derived from autologous bone marrow and from the donor bone marrow. The detection of persistent chimerism is the only reliable evidence for bone marrow or hematopoietic stem cell transplant "take". For sex-mismatched transplants evidence of chimerism can be obtained by FISH with Y chromosome probes (some leukocytes will be positive, others negative). For sex matched grafts molecular studies, such as the determination of the number of variable number of tandem repeats or VNTR, are used. All the other listed parameters would be indicative of functional restoration but do not identify the source of the regenerating cells.

Note about VNTR determination
On some human chromosomes, a short sequence of DNA has been repeated a number of times. In any particular chromosomes the repeat number may vary from one to thirty repeats. Since these repeat regions are usually bounded by specific restriction enzyme sites, it is possible to cut out the segment of the chromosome containing this variable number of tandem repeats or VNTR's, run the total DNA on a gel, and identify the VNTR's by hybridization with a probe specific for the DNA sequence of the repeat.



Shown to the left at the top are the chromosomes of the two parental individuals of the pedigree below. The first individual has one chromosome with 4 repeated sequences and one chromosome with 6 repeated sequences. The other individual has one chromosome with 3 repeated sequences and one chromosome with 5 repeated sequences.

At the bottom of the figure is a pedigree of the mating between these two individuals and their four children. The DNA of each of the individuals has been analyzed for the VNTR repeat number and the gels are show below each individual along with the genotype for each individual. Notice that each of the six people are distinguishable from each other by the VNTR's at this one genetic locus. If several VNTR loci were used, the uniqueness of each individual would become even more distinct

9. (D) is correct. Commonly, since the recipients of hematopoietic stem cells have undergone chemotherapy and radiotherapy to eliminate all the cancerous cells and, as a consequence, all of their normal stem cells, these patients frequently develop a transient anemia which must be treated by red cell transfusion. Incidentally, some patients do receive tetanus immunization or tetanus booster shots, but this is performed at a later stage, to determine whether the stem cell transplant has established itself. Interleukin-2 therapy is not known to be of value in assuring the success of a hematopoietic stem cell transplant. Obviously, some patients may need psychotherapy after having gone through such a traumatic procedure, but not all patients require this. Megadoses of folic acid are unlikely to accelerate bone marrow regeneration.

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Data Sheet B
Patient follow-up

The patient was started on Gleevec according to a standard treatment regimen. In the following weeks the patient's blood WBC count decreased and stayed in the low normal range (4,500 cells/µl). At 11 months after beginning Gleevec therapy, the patient was carefully evaluated. The spleen could not be palpated and the WBC number, platelet counts, and hemoglobin levels were found to be normal.

Another bone marrow biopsy was performed at 13 months and the cellular morphology of the marrow was normal. However, the Philadelphia chromosome was found in 40% of the cells. It was concluded that Gleevec therapy was not likely to completely suppress the leukemic clone.

During a clinic visit you mention the possibility of a hematopoietic stem cell transplant (HSCT).

The patient indicates that he has two siblings, a brother and a sister. HLA typing of the patient, his parents, and his siblings is performed (see below).

Family Study for the Patient, his parents, and two siblings

	HLA-A	HLA-B	HLA-C	HLA-DR	HLA-DQ
Patient	2	47	6	7	2
	3	27	2	4	8
Father	2	47	6	7	2
	3	62	3	11	5
Mother	1	8	7	17	2
	3	27	2	4	8
Sibling 1	3	62	3	11	5
	3	27	2	4	8
Sibling 2	1	8	7	17	2
	2	47	6	7	2

What do you conclude from this data? Is there a family member who is an HLA match with the patient?

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BOOK C

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   A. Describe the sources of hematopoietic stem cells.
   B. Describe how a stem cell donor is chosen.

2. Describe how the outcome (success, failure) of an HSCT is determined.
   A. Define the goals of HSCT transplantation.
   B. Describe how the corrective effect of an HSCT is determined.

3. Describe the immunological barriers that must be considered in doing an HSCT.
   A. Describe host-versus-graft disease (HVGD) and graft-versus-host disease (GVHD).
   B. Describe how an immune response can have a detrimental effect on the success of an HSCT.
   C. Explain how the immune system of the graft recipient can be manipulated to prevent GVHD.
   D. Explain how a graft-versus-leukemia reaction can be of benefit to the patient.

4. Describe the risks associated with HSCT.
   A. Know the success rate of HSCT in curing a disease such as chronic myelogenous leukemia.
   B. Describe the possibility of recurrent disease, infection, cancer, and GVHD and how these conditions may affect the success of the HSCT.

5. Describe the pathogenesis and clinical manifestations of GVHD.

Booklet C
Pretest Correct Answers

3. (E) is correct. One of the major barriers to successful hematopoietic stem cell transplantation is the antigens coded for by the major histocompatibility gene complex. Identical twins have identical major histocompatibility gene complexes and therefore have identical major histocompatibility complex molecules. Individuals such as a parent, first cousin, or unrelated individual, are not likely to be perfect matches and therefore are not ideal donors for hematopoietic stem cells. It must be noted that even when a donor is apparently identical, typing has only been performed for a relatively small number of genetic markers and there is ample room for differences in those that have not been typed. On the positive side, typing includes those markers that have a stronger influence in the outcome of a graft.

6. (D) is correct. Clearly, patients who have undergone the required combination of chemotherapy and radiotherapy prior to receiving the stem cell transplant are at increased risk for infection and sometimes must be hospitalized in special clean rooms so as to minimize the chances of exposure to infectious agents. In addition, the hematopoietic stem cell transplant may often contain immunologically competent T lymphocytes, which may attack the recipient's cells causing GVHD. It is known that there is an increased risk in recipients of hematopoietic stem cell transplants of cancer. This risk is thought to be associated with the chemotherapy and radiotherapy, which these patients receive prior to receiving the transplants. However, the malignancies originate in host tissues, not on grafted cells. Finally, not every hematopoietic stem cell transplant is successful; some of these transplants fail to thrive and localize graft in the bone marrow. Others are rejected by the host’s immune system.

10. (C) is correct. The greatest risk associated with being a donor of hematopoietic stem cells is infection due to the invasion of the bone marrow by a needle in order to harvest the stem cells. Even so, infection is rare in the donors. Anemia is not encountered but in the rarest circumstance because the donors are carefully evaluated for their health and capacity of their bone marrow to produced adequate numbers of red and white blood cells for their own bodies. Bone marrow failure has not been recorded to occur. Transmission of leukemia is not possible, because there is no previous contact of the needle used to harvest the bone marrow with the patient’s tissues. Lymphadenopathy or enlargement of the lymph gland also has not been reported.

Transplantation Immunology

Introduction

Thousands of transplants of organs, tissues, and cells are performed throughout the world annually. Transplantation of bone marrow cells or hematopoietic stem cell transplantation (HSCT) is performed to correct failures in the production of red or white cells, to repair immune deficiencies, and as a means of replacing malignant cells which have taken over the bone marrow. The major barrier to organ, tissue, and cell transplantation in humans is the immune response to the graft or, in the case of HSCT, the immune response of the graft against the recipient.

This POPS leads to a discussion on the indications and problems associated with HSCT or allogeneic bone marrow transplantation (ABMT) in a patient with acute leukemia. Approximately 70% of the patients undergoing ABMT for the treatment of certain types of leukemia live at least five years. A major barrier to the success of HSCT is the fact that the patient's immune system can reject the donor stem cells or that lymphocytes in the HSCT will attack (reject) the recipient. Donor selection is very important in order to prevent or minimize immune reactions of host against graft and graft against host. It is important to find the best possible histocompatibility match between the donor and the patient.

There are at least seven genes in the major histocompatibility complex (MHC), located on chromosome 6, which code for HLA antigens. Each of these seven genes is polymorphic meaning that there are many different versions of each gene which differ from the others by a different sequence of nucleotides. Each of these gene variants, called **alleles**, code for an antigenically different HLA antigen. In the human population there are many, many thousands of different MHC gene combinations or **genotypes**, as they are called. Faced with this huge variability, it is very difficult, but not impossible, to find two human beings who have identical HLA antigens by searching in the population at random. Since HLA antigens are genetically determined, it is often possible to find individuals who are HLA identical, or matched, within a family.

The HLA antigens that receive the most attention are HLA-A, HLA-B and HLA-DR, however, HLA-C and HLA-DQ may also play an important role. Since each person carries a chromosome six of maternal and paternal origin there are two copies of each HLA gene all of which are expressed (co-dominant expression). Therefore, a “perfect” match is called a six-antigen match (2 HLA-A, 2 HLA-B, and 2 HLA-DR), although as previously stated there are other antigens that may be important. The HLA genes on a particular chromosome 6 constitute a “haplotype” (derived from haploid genotype). Each person has a maternal and a paternal haplotype based on which of the parent’s chromosome 6s were inherited.

The most likely place to find an HLA match between two people is among full-blooded siblings. According to Mendelian inheritance principles, one has a 25% chance of inheriting the same HLA molecules as a sibling, a 25% chance of inheriting none of the same HLA molecules as a sibling and a 50% chance of inheriting half of the same HLA molecules as a sibling. When HSCT is considered, the first thing is to do a “family study”. The patient and all available siblings and parents are HLA typed. This allows for identification of potential “six-antigen” matches among the siblings and analysis of the segregation patterns of the HLA molecules. The segregation patterns are obtained from determining which of the parental haplotypes are inherited. Each sibling from a given couple should have one haplotype from each parent.

Introduction (ctd.)

In the example shown below, five of the seven MHC genes of the two families were determined. Each HLA antigen coded for by an MHC gene is identified by a number. Since we inherit one set of genes from each of our parents, we all have two versions of each MHC gene and thus there are two numbers given for each gene. Because data from both parents and two siblings was available, it was possible to establish the genotypes for the different family members. Discuss the interpretation of the HLA typing data below within the group before turning this page.

HLA Genotypes

		Family 1					Family 2				
Family Member	HLA Gene	A	B	C	DR	DQ	A	B	C	DR	DQ
Mother		1	5	45	10	21	15	25	40	51	55
		2	6	46	11	22	16	26	41	52	56
Father		3	7	47	12	23	17	27	42	53	57
		4	8	48	13	24	18	28	43	54	58
Child 1		1	5	45	10	21	15	26	40	52	55
		4	8	48	13	24	17	27	43	53	57
Child 2		2	6	46	11	22	15	26	40	52	55
		3	7	47	12	23	17	27	43	53	57

Data Sheet C
Histocompatibility testing data (continued from Data set B)

Family Study for the Patient, his parents, and two siblings

	HLA-A	HLA-B	HLA-C	HLA-DR	HLA-DQ
Patient	2	47	6	7	2
	3	27	2	4	8
Father	2	47	6	7	2
	3	62	3	11	5
Mother	1	8	7	17	2
	3	27	2	4	8
Sibling 1	3	62	3	11	5
	3	27	2	4	8
Sibling 2	1	8	7	17	2
	2	47	6	7	2

The results of HLA typing indicate that both of the patient's siblings are only half matches with the patient meaning that neither the brother nor the sister is compatible with the patient. You decide to search for an unrelated donor who is a better match than either one of the siblings. The patient indicates that he would like to proceed with this transplant immediately. You inform him that a search for a matched, unrelated donor through the National Marrow Donor Program (NMDB) in Minneapolis, MN can take at least four months. You also inform the patient that he must be withdrawn from the interferon alpha therapy for at least three months before the HSCT is performed. You tell him that receiving an HSCT from a donor who is not HLA matched can result in the graft being rejected by the patient's immune response or donor lymphocytes in the HSCT could attack the recipient causing a condition called graft-versus-host disease (GVHD).

The database in the computers at the NMDP program contains the MHC types on at least 3 million potential donors in the United States. There are donor networks throughout the world, which increase the possibility of finding a matched, unrelated donor. The following four individuals are considered as potential donors:

NMDP Potential Donors for Patient

NMDP #	A/S/R*	HLA-A	HLA-B	HLA-DR	HLA-DQ
3243-5423-5	55/F/W	2	47	7	ND
		3	27	7	ND
4328-7895-7	33/M/B	2	47	7	2
		3	62	11	3
8493-0983-2	62/F/W	2	47	7	2
		3	62	11	8
3423-5646-1	45/F/W	2	47	7	ND
		3	62	11	ND

*Age/Sex/Race

Which one of the donors would you select for this patient?

Data sheet C
Histocompatibility testing data (continued)

Donor 3243-5423-5 appears to be the best match of the four candidates, and is also a better match than either of the patient's siblings.

The Gleevec therapy is stopped and the patient is counseled regarding the possible outcomes of HSCT. An HSCT can fail to engraft; this occurs in approximately 10% of the cases. Second HSCTs from the same donor are not always available and a second graft may also fail. A major concern and risk of unrelated donor HSCT is the risk of GVHD. This is an immunologic response in which cells in the graft attack the host. GVHD can be mild to severe; when severe, it can be life threatening.

You remind the patient that untreated CML is a uniformly fatal disease. Patients who exhibit a good response as far as the disappearance of the Philadelphia chromosome positive cells have a good prognosis; five-year survival is 70% for patients in whom HSCT is successful.

In preparation for the HSCT, the patient is given high doses of chemotherapy and total body irradiation. When the immunosuppressive treatment of the recipient is about to be completed, bone marrow or peripheral blood stem cells are harvested from the donor. To harvest bone marrow the donor is placed under general anesthesia and marrow is collected from the posterior-superior iliac crests. An alternative is to administer G-CSF to the donor to mobilize stem cells from the bone marrow and harvest peripheral blood, from which a stem cell enriched leukocyte preparation can be easily obtained.

Discuss how can stem cells be separated from the donor's bone marrow or peripheral blood leukocyte preparation.

Transplantation Immunology

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BOOK D

Note to Students: The fundamental purpose of all activities in the health-care professions is to help other people. Like all behaviors, helping behavior becomes more effective and natural with practice. This workbook enables you to practice by helping your fellow students to learn basic science. Your skill at helping your fellow students should relate to your ability to help your patients in the future. This is a *Patient-Oriented Problem-Solving ("POPS")* workbook designed for four students. Before beginning this session, you should have (a) studied the objectives designed to prepare you for it, (b) taken the pretest, and (c) reviewed the topics listed at the end of the pretest. Now, each of you should take one of the four color-coded booklets and follow the directions in it. If your group has only three students, one of you should take two booklets. Leave the remainder of the workbook intact until you are given further instructions.

Transplantation Immunology

Introduction to the Patient-Oriented Problem-Solving (POPS) System

This is a Patient-Oriented Problem-Solving activity. The purposes are

1. To help you learn how to apply your basic science knowledge to the solution of clinical problems

2. To help you learn how to better use sources (i.e., textbooks and peers) that will be available to you throughout your career

This activity consists of four phases. First, you will review the attached set of objectives, do background reading on the topics to be covered, and complete the pretest on your own. In the second phase, you will join three other students and review the pretest answers in an "open-book" discussion. In the third phase, the group will solve patient-oriented problems. Information exchange and group interactions are keys to the success of this phase. This process will allow you to teach your fellow students and, at the same time, learn from them. Finally, you will take a posttest, individually, which will enable you to assess your progress.

Transplantation Immunology

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Transplantation Immunology

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Booklet D
Pretest Correct Answers

4. (C) is correct. Technically performing a hematopoietic stem cell transplant is one of the simplest transplantation procedures. The hematopoietic stem cells are infused into a patient's blood stream using an intravenous infusion system. The surface adhesion molecules of the transfused stem cells will mediate their migration to the appropriate territory(ies) No invasive surgery or invasive procedures are involved.

7. (B) is correct. When a graft containing immunocompetent cells is placed into an immunocompromised host, the transplanted cells can recognize as non-self the host antigens. In response to these antigenic differences, the donor T lymphocytes become activated, proliferate and differentiate into helper and effector cells that attack the host cells and cause GVHD. The crucial role played by the donor T cells in GVHD is demonstrated by the fact that their elimination from a bone marrow graft avoids the reactions, although the therapeutic benefit is also significantly reduced. However, as the GVHD evolves, the majority of the cells infiltrating the different tissues affected by the GVH reaction are of host origin and include T and B lymphocytes as well as monocytes and macrophages. The proliferation of host cells is probably a result of the release of high concentrations of non-specific mitogenic and differentiation factors by activated donor T lymphocytes. Granulocytes are rarely seen in the cellular infiltrates characteristic of GVHD.

Data sheet D

Stem cell isolation

Stem cells can be isolated from the bone marrow or the peripheral blood leukocyte preparation by flow cytometry or using magnetic monoclonal antibodies. The stem cells are by their reactivity with CD34 monoclonal antibodies. When using “magnetic” monoclonal CD34 antibodies the stem cells with bound antibodies are retained on a container exposed to a magnetic field. After extensive washing of the container, the magnetic field is removed and $CD34^+$ stem cells are recovered. Some groups inject directly the isolated stem cells while others expand the $CD34^+$ stem cells *ex vivo* with cytokine cocktails and then infuse them into the patient intravenously.

However, most groups prefer to use unfractionated $CD34^+$-enriched leukocyte preparations (buffy coat) obtained directly from the donor's blood for transplantation, because the patients seem to recover more quickly from the effects of immunoablation with this protocol.

Data sheet D
Post-graft evolution (continued)

Approximately 10 days after the HSCT, the patient becomes febrile. Blood cultures are ordered but are negative. The patient was treated with antibiotics and the fever abated. One week after transplantation the patient was given G-CSF to enhance granulocyte production. Fourteen days after HSCT, the patient's white blood count began to increase compared to pretransplant count. The patient was given red blood cell and platelet transfusions twice weekly during the first 10 weeks after HSCT. At one month after HSCT a bone marrow biopsy revealed a relatively vacant marrow (the marrow was hypocellular). Philadelphia chromosome positive cells were not found. At approximately 36 days after HSCT the patient was discharged from the hospital and continued to receive antibiotics plus intravenous gamma globulin (IVIg) and Cyclosporine.

What do you believe was the cause of this patient's fever?

Why were antibiotics prescribed to this patient at the time of discharge?

Why was the patient treated with IVIg and cyclosporine?

Data sheet D

Post-graft evolution (continued)

When the patient developed fever he needed to be given antibiotics covering enteric Gram negative and skin Gram positive organisms, those being the most likely sources of bacteremia. Several antibiotics or combinations of antibiotics can be used, such as a carbenapem (imipenem) by itself or in association with vancomycin, or an extended spectrum penicillin (e.g. piperacillin or ticarcillin) in association with an aminoglycoside. At discharge the patient was given ciprofloxacin, to lower the intestinal bacterial load, and IVIg (which contains antibodies to the most common pathogens) to prevent the most prevalent infections in immunosuppressed patients. Cyclosporine was given to prevent the development of a severe form of GVHD.

30 days after the HSCT the patient came in for a routine clinic follow-up visit and a nurse noticed a rash on the patient's lower back and several lesions on the forearms and palms of the hands, illustrated in figure 1. A biopsy of the affected skin is shown in figure 2.

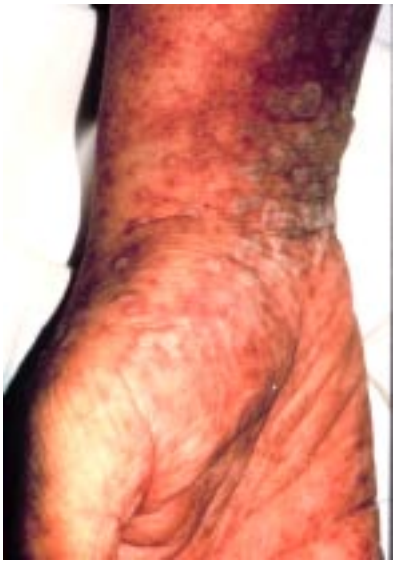

Photo courtesy of Dr John Maize

Figure 1

Figure 2

How do you interpret this biopsy?

Data sheet D
Post-graft evolution (continued)

The skin biopsy shows degeneration of the basal layer of the epidermis and a superficial perivascular mononuclear cell infiltrate at the dermo-epidermal junction with invasion of the epidermis by the infiltrating mononuclear cells. High power examination showed apoptotic squamous cells with adjacent lymphocytes in the epidermis. A diagnosis of GVHD was made. In addition to systemic administration of immunosuppressive drugs, topical therapy with corticosteroid cream was prescribed and alleviated the rash. Approximately one month later, the patient came to the clinic complaining of abdominal cramping and two days later developed diarrhea with liquid and bloody stools. Endoscopic examination of the gastrointestinal tract including the colon revealed inflammation and mucosal changes. Several biopsies of the gut were taken and the patient was admitted to the hospital. Figure 3 illustrates the findings observed in one of these biopsies (low power).

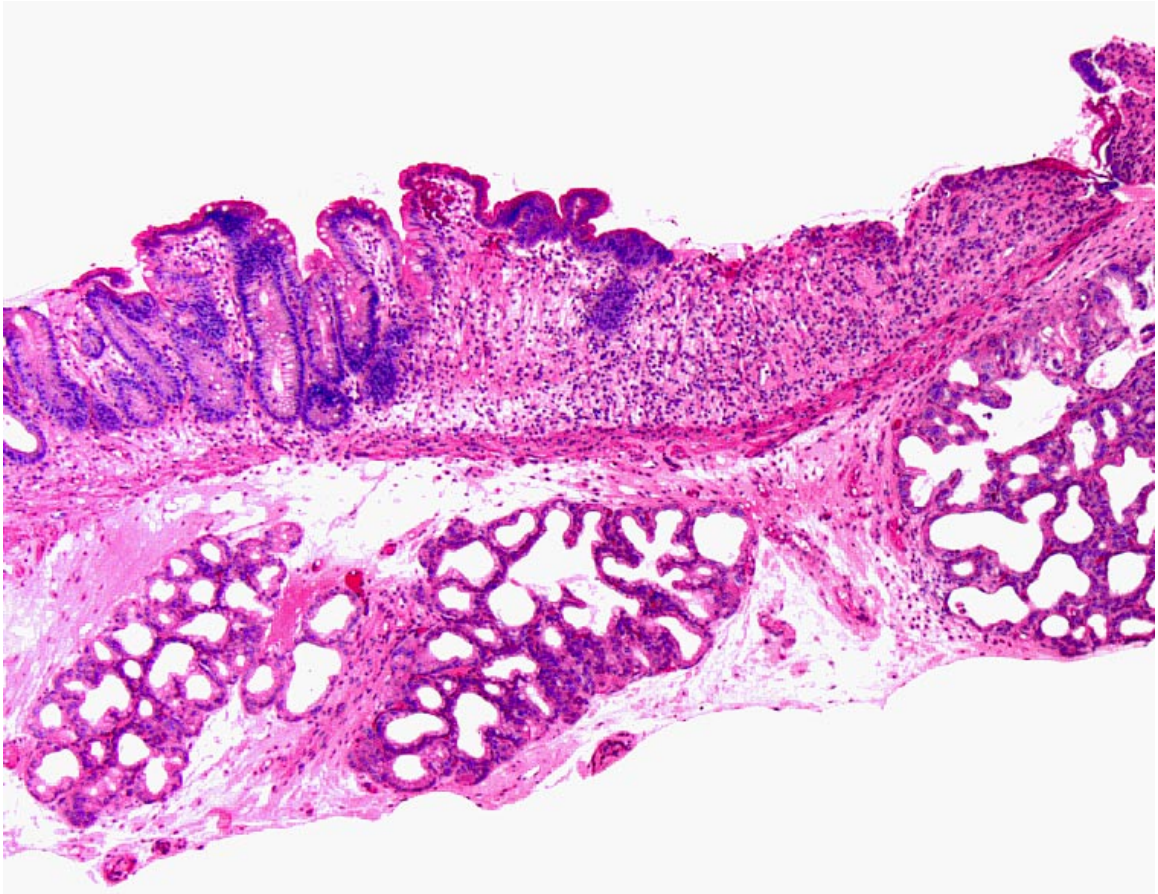

Photo courtesy of Dr. David Lewin

Figure 3

How do you interpret this biopsy? What would you do next?

Data sheet D
Post-graft evolution (continued)

Low power microscopic view of a duodenal biopsy showed a decreased villous to crypt ratio, glandular loss and mucosal erosion. Brunner's glands with dilation are present beneath the *muscularis mucosa*. A submucosal diffuse mononuclear cell infiltrate, confirmed to be predominantly constituted by lymphocytoid cells on high power examination, was also apparent. The gut biopsy was compatible with a diagnosis of systemic GVHD. It was decided to prescribe additional immunosuppressive therapy. The patient was treated with intravenous methylprednisolone and improved rapidly over the next three days. Approximately 10 days later the patient was discharged from the hospital with a prescription for prednisone, which was to be tapered according to the usual protocol. One hundred days after HSCT the patient reported that he felt well. On one clinic visit at 210 days after HSCT further treatment with oral prednisolone for a skin rash was prescribed. One year after the transplant the patient was able to return to work.

Discuss the pathogenesis of GVHD and other therapeutic alternatives not yet considered in this patient.

Transplantation Immunology

Data sheet D

Closing comments

Whenever a patient receives a graft of an organ rich in immunocompetent cells, there is a risk that **graft-versus-host disease (GVHD)** may develop. GVHD is a significant problem in infants and children with primary immunodeficiencies in whom a bone marrow transplant is performed with the goal of reconstituting the immune system, as well as in adults receiving a bone marrow transplant as part of a therapeutic protocol for aplastic anemia or for a hematopoietic malignancy, because the immune system of these patients is ablated as preparation for the graft. The probability of developing GVHD is greatest in the two-month period immediately following transplantation.

When a graft containing immunocompetent cells is placed into an immunoincompetent host, the transplanted T cells can recognize host-derived peptides as non-self. As a consequence the donor T lymphocytes become activated, proliferate and differentiate into helper and effector cells that attack the host cells and tissues, producing the signs and symptoms of GVHD. The crucial role played by the donor T cells in GVH is demonstrated by the fact that their elimination from a bone marrow graft avoids the reactions. However, as the GVH evolves and reaches its highest intensity, the majority of the cells infiltrating the different tissues affected by the GVH reaction are of host origin and include T and B lymphocytes as well as monocytes and macrophages. The proliferation of host cells is probably a result of the release of high concentrations of non-specific mitogenic and differentiation factors by activated donor T lymphocytes.

The initial proliferation of donor T cells takes place in lymphoid tissues, particularly in the liver and spleen (leading to hepatomegaly and splenomegaly). Later, at the peak of the proliferative reaction, the skin, liver and intestinal walls are heavily infiltrated leading to severe skin rashes or exfoliative dermatitis, hepatic insufficiency, and severe diarrhea or even intestinal perforation. The splenic involvement results in a loss of function not unlike that seen in splenectomized patients. The patients often develop *Streptococcus pneumoniae* bacteremia and antibiotic prophylaxis may be necessary.

All immunosuppressive drugs used in the prevention and treatment of rejection, including cyclosporine, tacrolimus, sirolimus, and mycophenolate mofetil, have been used for treatment of the GVH reaction. In addition, cytokine modifiers, such as infliximab, and thalidomide**,** the tranquilizer drug that achieved notoriety due to its teratogenic effects, have been also used with mixed success for the control of chronic GVH unresponsive to traditional immunosuppressants.

In leukemic patients receiving allogeniec bone marrow or stem cells as part of the treatment for their disease GVHD can be prevented by **T cell depletion** of the graft. This can be achieved by pre-treatment of the bone marrow with anti-lymphocyte/thymocyte immunoglobulin, or with monoclonal antibodies reacting with T cells (e.g., anti-CD3) or by using **autologous stem or allogeneic umbilical cord stem cells**(stem cells obtained from cord blood after delivery). In all cases the rate of cure is lower. A low grade, controllable GVHD is associated with better outcomes in leukemic patients, perhaps as a result of the elimination of leukemic cells by the grafted lymphocytes (graft-versus-leukemia effect).

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When a graft containing immunocompetent cells is placed into an immunoincompetent host, the transplanted T cells can recognize host-derived peptides as non-self. As a consequence the donor T lymphocytes become activated, proliferate and differentiate into helper and effector cells that attack the host cells and tissues, producing the signs and symptoms of GVHD. The crucial role played by the donor T cells in GVH is demonstrated by the fact that their elimination from a bone marrow graft avoids the reactions. However, as the GVH evolves and reaches its highest intensity, the majority of the cells infiltrating the different tissues affected by the GVH reaction are of host origin and include T and B lymphocytes as well as monocytes and macrophages. The proliferation of host cells is probably a result of the release of high concentrations of non-specific mitogenic and differentiation factors by activated donor T lymphocytes.

The initial proliferation of donor T cells takes place in lymphoid tissues, particularly in the liver and spleen (leading to hepatomegaly and splenomegaly). Later, at the peak of the proliferative reaction, the skin, liver and intestinal walls are heavily infiltrated leading to severe skin rashes or exfoliative dermatitis, hepatic insufficiency, and severe diarrhea or even intestinal perforation. The splenic involvement results in a loss of function not unlike that seen in splenectomized patients. The patients often develop *Streptococcus pneumoniae* bacteremia and antibiotic prophylaxis may be necessary.

All immunosuppressive drugs used in the prevention and treatment of rejection, including cyclosporine, tacrolimus, sirolimus, and mycophenolate mofetil, have been used for treatment of the GVH reaction. In addition, cytokine modifiers, such as infliximab, and thalidomide**,** the tranquilizer drug that achieved notoriety due to its teratogenic effects, have been also used with mixed success for the control of chronic GVH unresponsive to traditional immunosuppressants.

In leukemic patients receiving allogeniec bone marrow or stem cells as part of the treatment for their disease GVHD can be prevented by **T cell depletion** of the graft. This can be achieved by pre-treatment of the bone marrow with anti-lymphocyte/thymocyte immunoglobulin, or with monoclonal antibodies reacting with T cells (e.g., anti-CD3) or by using **autologous stem or allogeneic umbilical cord stem cells**(stem cells obtained from cord blood after delivery). In all cases the rate of cure is lower. A low grade, controllable GVHD is associated with better outcomes in leukemic patients, perhaps as a result of the elimination of leukemic cells by the grafted lymphocytes (graft-versus-leukemia effect).

Transplantation Immunology

Data sheet D

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Transplantation Immunology

Posttest

1. A patient with aplastic anemia is given a hematopoietic stem cell transplant from a matched, but unrelated bone marrow donor. At 35 days after the transplant the patient exhibits signs of graft-versus-host disease. One of the ways in which this disease could have been prevented in this patient is by:
   A. eliminating B lymphocytes from the donor marrow
   B. eliminating the patient's major histocompatibility complex molecules with intravenous proteolytic enzymes
   C. treating the patient with interleukin-2 after the transplant
   D. eliminating T lymphocytes from the donor bone marrow
   E. eliminating all CD34+ cells from the donor marrow

2. The major drawback of the depletion of T cells from a preparation of allogeneic stem cells to be transplanted to a leukemic patient is:
   A. elimination of the graft vs. leukemia effect
   B. higher probability of graft rejection
   C. no effect on the incidence of graft-versus-host disease
   D. prolonged post-graft immunosuppression
   E. technical difficulty

3. Patients who receive hematopoietic stem cell transplants from unrelated donors are frequently treated prophylactically with an immunosuppressive drug such as Cyclosporine. The basis for giving an immunosuppressive drug to a patient whose immune system has been suppressed by chemotherapy and total body irradiation is that Cyclosporine administration:
   A. minimizes or prevents the development of graft-versus-host disease.
   B. stimulates the growth of the hematopoietic stem cells in the recipient.
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4. An **early** indication that a hematopoietic stem cell transplant has been successful is:
   A. a normal CD4:CD8 T lymphocyte ratio in the blood of the recipient.
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5. Biopsy of a skin rash site and the gut wall epithelium in a patient who has received a hematopoietic stem cell transplant is used to confirm the diagnosis of graft-versus-host disease. The presence of mononuclear cell infiltrates in the skin and in the gut epithelium indicates that graft-versus-host disease is:
   A. a cell-mediated immune reaction
   B. an antibody-mediated immune reaction
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Posttest (continued)

6 Which one of the following organ or tissue transplants are LEAST likely to cause a graft-vs.-host reaction?

A. Bone marrow
B. Heart-lungs
C. Kidney
D. Liver
E. Small intestine

7. Which of the following is least likely to be a complication of hematopoietic stem cell transplantation:

A. autoimmune hemolytic anemia
B. graft rejection or failure of the stem cell graft to survive
C. graft-versus-host disease
D. opportunistic infection
E. post transplant immune deficiency

8. Which of the following determinations will give better information about the achievement of **immunological reconstitution** after stem cell or bone marrow transplant?

A. Development of hypergammaglobulinemia
B. Establishment of a state of chimerism
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9 Athymic nude mice are transplanted with bone marrow from genetically unrelated and immunocompetent Balb/c mice. Identify in the following chart the most likely combination of results seen in the grafted mice.

Nude recipient / Balb/c donor		
	B.M. graft	**Systemic effects**
A	Rejected	None
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E	Accepted	Lymphomas, infections

10. Which of the following membrane markers is the basis for the separation of human stem cells from bone marrow aspirates?

A. CD10 (CALLA)
B. CD19
C. CD25
D. CD34
E. CD40

Transplantation Immunology

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Transplantation Immunology

Posttest Correct Answers

1. (D) is correct. It is the immunocompetent T cells in the allogeneic donor marrow that mediate the graft-versus-host disease. Because differentiated T cells do not express CD34, stem cell transplantation is associated with a lower risk of GVHD than transplantation of unfractionated bone marrow. Autologous stem cells or cord blood stem cells are even less likely to contain autoreactive T cells able to induce GVHD, but the complete lack of alloreactive T cells abrogates the graft-vs. leukemia effect as well and the rates of cure become lower. B lymphocytes do not mediate graft-versus-host disease. Obviously, removing the patient's major histocompatibility complex molecules from all of the cells in the body would not be possible and intravenous injection of proteolytic enzymes would be toxic in any case. Treatment of the recipient with interleukin-2 might actually increase the severity of graft-versus-host disease and thus would be avoided. Clearly, it is the hematopoietic stem cells in the donor marrow that are needed the effect the cure in a patient with chronic myelogenous leukemia. Thus, removal of the stem cells would be counter-productive.

2. (A) is correct. **T cell depletion** of the graft can reduce significantly the incidence of GVHD. The major problem is that the clinical outcome in leukemic patients is worse, with high mortality. In part this is due to the fact that a low grade, controllable GVHD is associated with better outcomes in leukemic patients, perhaps as a result of the elimination of leukemic cells by the grafted lymphocytes (graft-versus-leukemia effect). In addition, the transplantation of T cell depleted bone marrow into immunosuppressed adults may result in a persistent state of severe immunodeficiency. These data suggest that the T cells facilitate the engraftment of the donor stem cells within the host bone marrow, although the mechanism for this is not understood.

3. (A) is correct. Prophylaxis with Cyclosporine, an inhibitor of T lymphocyte activation, is used to offset the development of graft-versus-host disease in patients receiving allogeneic hematopoietic stem cell transplants. Cyclosporine is not known to stimulate stem cell growth factor(s) production nor does it suppress the growth of leukemia cells.

4. (D) is correct. As in the case discussed in this POPS, an increase in the number or normal appearing white blood cells in the blood of the recipient which occurs at three weeks to one month after transplantation is taken as an indicator of the success of the hematopoietic stem cell transplant. The hematopoietic stem cells populate the recipient's bone marrow cavities differentiating into blood leukocytes, which then appear as mature cells in the peripheral blood. Neither the number of T lymphocytes nor the CD4:CD8 T lymphocyte ratio can be used as an indicator of the success of a hematopoietic stem cell transplant. Generally the antibody titers to commonly encountered infectious agents are lowered by the chemotherapy and radiotherapy performed immediately prior to transplantation. However, the antibody titers do not automatically rebound following a successful transplant. A patient's hematocrit does not rise in the days immediately following transplantation, but requires several weeks to months to return to normal levels. Similarly, the disappearance of graft-versus-host disease mediated by T lymphocytes in the donor marrow could not be taken as an indicator of success of the transplant. The disappearance of graft-versus-host disease may indicate that the immunosuppressive drug has successfully treated this disease.

Posttest Correct Answers (continued)

5. (A) is correct. Graft-versus-host disease is largely, if not entirely, mediated by immunocompetent T lymphocytes that are present in the hematopoietic stem cell transplant from the donor. These T lymphocytes become activated by the alloantigens of the transplant recipient, mature into activated, antigen-specific T lymphocytes, and localize to sites such as the skin, gut epithelium, liver, and elsewhere in the body where they initiate destruction of recipient cells. The activated graft cells participating in the reaction release large amounts of cytokines, some of which attract and activate monocytes and macrophages, also abundant in the infiltrates. Residual populations of recipient immune cells, often autoreactive, become activated as well and play a significant pathogenic role in GVHD. Antibodies do not appear to play a major or significant role in graft-versus-host disease; natural killer cells are also not major factors in this disease.

6 (C) is correct. The likelihood of developing a GVH reaction is minimal when solid organs with minimal endogenous lymphoid tissue, such as the kidney and the heart, are grafted. However, the likelihood increases in a heart-lung transplant due to the lung-associated lymphoid tissues.

7. (A) is correct. Autoimmune hemolytic anemia in a hematopoietic stem cell transplant recipient may occur during GVHD if autoreactive clones are activated, but the frequency of this event is low. The other answers, such as graft rejection or failure of the graft to take, graft-versus-host disease, immune deficiency after transplant, and opportunistic infection are all observed with considerable higher frequency following hematopoietic stem cell transplantation.

8. (E) is correct. The induction of an active immune response by immunization is considered as evidence of immunological reconstitution. It is likely that a state of chimerism, in which cells of both donor and recipient origin repopulate the bone marrow without reacting to each other is the best possible outcome for this type of transplant, but by itself does not prove that there has been total reconstitution of the immune system. The same is true for other positive outcomes, such as normalization of the white blood cell count and of the $CD4^+$ T cell count. On the other hand, polyclonal hypergammaglobulinemia is often seen in cases of GVHD and reflects the indiscriminate activation of B cells, not associated with recovery of the ability to respond to a specific challenge.

9 (D) is correct. Athymic mice will lack T cells and will not be able to reject the graft, however, the grafted T cells will be able to mount a GVHD. The three major manifestations of GVHD are splenomegaly, diarrhea, and wasting. Lymphomas and infections are more prevalent in immunocompromised animals, but not as a consequence of the bone marrow transplant.

10. (D) is correct. CD34 is a marker that allows selection of stem cells from the bone marrow, peripheral blood, or cord blood. It can be used for selection of either autologous or allogeneic stem cells. The several varieties of stem cell grafting are becoming the approach of choice for the treatment of hematopoietic malignancies. CD10 is a marker of leukemic cells, which can be used to "purge" them from autologous bone marrow, for example. CD19 is a mature B cell marker. CD25 is a marker associated with the IL-2 receptor, upregulated in activated lymphocytes. B cells express CD40 and its interaction with CD154 (CD40 ligand), expressed by activated helper T cells, delivers an important differentiation signal to the B cell.

Transplantation Immunology

Posttest Correct Answers

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8. (E) is correct. The induction of an active immune response by immunization is considered as evidence of immunological reconstitution. It is likely that a state of chimerism, in which cells of both donor and recipient origin repopulate the bone marrow without reacting to each other is the best possible outcome for this type of transplant, but by itself does not prove that there has been total reconstitution of the immune system. The same is true for other positive outcomes, such as normalization of the white blood cell count and of the $CD4^+$ T cell count. On the other hand, polyclonal hypergammaglobulinemia is often seen in cases of GVHD and reflects the indiscriminate activation of B cells, not associated with recovery of the ability to respond to a specific challenge.

9 (D) is correct. Athymic mice will lack T cells and will not be able to reject the graft, however, the grafted T cells will be able to mount a GVHD. The three major manifestations of GVHD are splenomegaly, diarrhea, and wasting. Lymphomas and infections are more prevalent in immunocompromised animals, but not as a consequence of the bone marrow transplant.

10. (D) is correct. CD34 is a marker that allows selection of stem cells from the bone marrow, peripheral blood, or cord blood. It can be used for selection of either autologous or allogeneic stem cells. The several varieties of stem cell grafting are becoming the approach of choice for the treatment of hematopoietic malignancies. CD10 is a marker of leukemic cells, which can be used to "purge" them from autologous bone marrow, for example. CD19 is a mature B cell marker. CD25 is a marker associated with the IL-2 receptor, upregulated in activated lymphocytes. B cells express CD40 and its interaction with CD154 (CD40 ligand), expressed by activated helper T cells, delivers an important differentiation signal to the B cell.

Transplantation Immunology

Posttest Correct Answers

1. (D) is correct. It is the immunocompetent T cells in the allogeneic donor marrow that mediate the graft-versus-host disease. Because differentiated T cells do not express CD34, stem cell transplantation is associated with a lower risk of GVHD than transplantation of unfractionated bone marrow. Autologous stem cells or cord blood stem cells are even less likely to contain autoreactive T cells able to induce GVHD, but the complete lack of alloreactive T cells abrogates the graft-vs. leukemia effect as well and the rates of cure become lower. B lymphocytes do not mediate graft-versus-host disease. Obviously, removing the patient's major histocompatibility complex molecules from all of the cells in the body would not be possible and intravenous injection of proteolytic enzymes would be toxic in any case. Treatment of the recipient with interleukin-2 might actually increase the severity of graft-versus-host disease and thus would be avoided. Clearly, it is the hematopoietic stem cells in the donor marrow that are needed the effect the cure in a patient with chronic myelogenous leukemia. Thus, removal of the stem cells would be counter-productive.

2. (A) is correct. **T cell depletion** of the graft can reduce significantly the incidence of GVHD. The major problem is that the clinical outcome in leukemic patients is worse, with high mortality. In part this is due to the fact that a low grade, controllable GVHD is associated with better outcomes in leukemic patients, perhaps as a result of the elimination of leukemic cells by the grafted lymphocytes (graft-versus-leukemia effect). In addition, the transplantation of T cell depleted bone marrow into immunosuppressed adults may result in a persistent state of severe immunodeficiency. These data suggest that the T cells facilitate the engraftment of the donor stem cells within the host bone marrow, although the mechanism for this is not understood.

3. (A) is correct. Prophylaxis with Cyclosporine, an inhibitor of T lymphocyte activation, is used to offset the development of graft-versus-host disease in patients receiving allogeneic hematopoietic stem cell transplants. Cyclosporine is not known to stimulate stem cell growth factor(s) production nor does it suppress the growth of leukemia cells.

4. (D) is correct. As in the case discussed in this POPS, an increase in the number or normal appearing white blood cells in the blood of the recipient which occurs at three weeks to one month after transplantation is taken as an indicator of the success of the hematopoietic stem cell transplant. The hematopoietic stem cells populate the recipient's bone marrow cavities differentiating into blood leukocytes, which then appear as mature cells in the peripheral blood. Neither the number of T lymphocytes nor the CD4:CD8 T lymphocyte ratio can be used as an indicator of the success of a hematopoietic stem cell transplant. Generally the antibody titers to commonly encountered infectious agents are lowered by the chemotherapy and radiotherapy performed immediately prior to transplantation. However, the antibody titers do not automatically rebound following a successful transplant. A patient's hematocrit does not rise in the days immediately following transplantation, but requires several weeks to months to return to normal levels. Similarly, the disappearance of graft-versus-host disease mediated by T lymphocytes in the donor marrow could not be taken as an indicator of success of the transplant. The disappearance of graft-versus-host disease may indicate that the immunosuppressive drug has successfully treated this disease.

Posttest Correct Answers (continued)

5. (A) is correct. Graft-versus-host disease is largely, if not entirely, mediated by immunocompetent T lymphocytes that are present in the hematopoietic stem cell transplant from the donor. These T lymphocytes become activated by the alloantigens of the transplant recipient, mature into activated, antigen-specific T lymphocytes, and localize to sites such as the skin, gut epithelium, liver, and elsewhere in the body where they initiate destruction of recipient cells. The activated graft cells participating in the reaction release large amounts of cytokines, some of which attract and activate monocytes and macrophages, also abundant in the infiltrates. Residual populations of recipient immune cells, often autoreactive, become activated as well and play a significant pathogenic role in GVHD. Antibodies do not appear to play a major or significant role in graft-versus-host disease; natural killer cells are also not major factors in this disease.

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Transplantation Immunology

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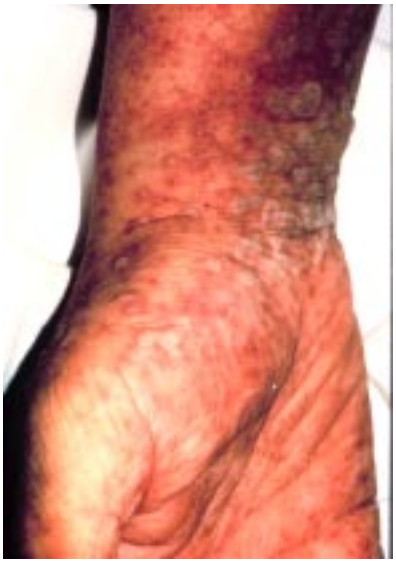

Figure 1

Figure 2

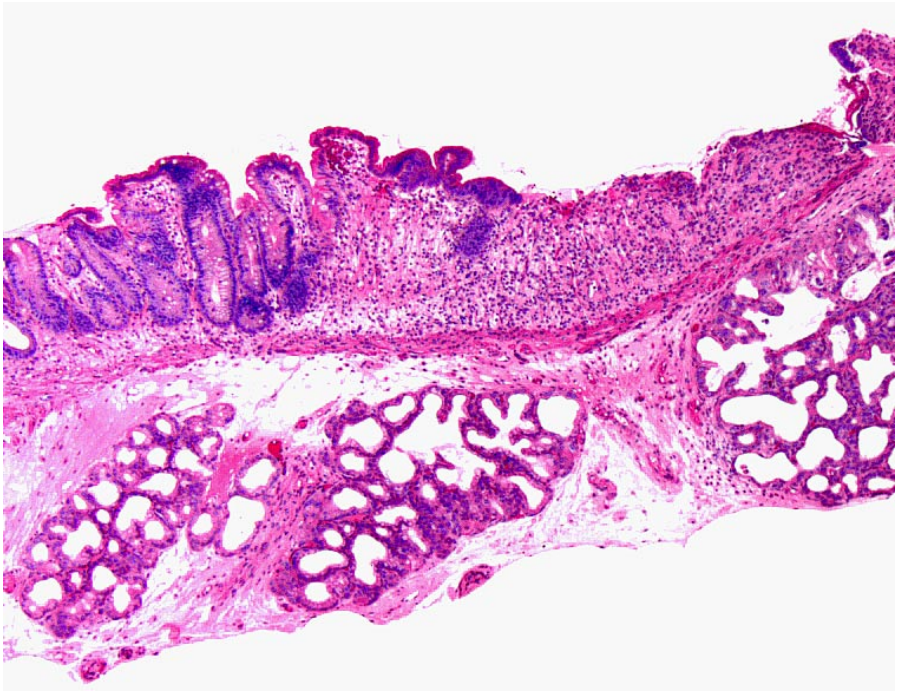

Figure 3